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**Methane emissions from two breeds of beef cows offered diets containing
barley straw with either grass silage or brewers' grains**

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Short title: Methane emissions from beef cows

Abstract

Increasing the concentration of dietary lipid is a promising strategy for reducing methane (CH₄) emissions from ruminants. This study investigated the effect of replacing grass silage with brewers' grains on CH₄ emissions of pregnant, non-lactating beef cows of two breeds. The experiment was a two x two factorial design comprising two breeds (LIMx, crossbred Limousin; and LUI, purebred Luing) and two diets consisting of (g/kg diet dry matter (DM)) barley straw (687) and grass silage (301, GS), or barley straw (763) and brewers' grains (226, BG), which were offered *ad libitum*. Replacing GS with BG increased the acid hydrolysed ether extract concentration from 21 to 37 g/kg diet DM. Cows (n=48) were group-housed in equal numbers of each breed across two pens and each diet was allocated to one pen. Prior to measurements of CH₄, individual dry matter intake (DMI), weekly BW and weekly body condition score were measured for a minimum of three weeks, following a four week period to acclimatise to the diets. Methane emissions were subsequently measured on one occasion from each cow using individual respiration chambers. Due to occasional equipment failures, CH₄ measurements were run over 9 weeks giving 10 observations for each breed x treatment combination (total n=40). There were no differences between diets for daily DMI measured in the chambers (9.92 vs. 9.86 kg/day for BG and GS, respectively; $P > 0.05$). Cows offered the BG diet produced less daily CH₄ than GS-fed cows (131 vs. 156 g/day; $P < 0.01$). When expressed either as g/kg DMI or kJ/MJ gross energy intake (GEI), BG-fed cows produced less CH₄ than GS-fed cows (13.5 vs. 16.4 g/kg DMI, $P < 0.05$; 39.2 vs. 48.6 kJ/MJ GEI, $P < 0.01$). Breed did not affect daily DMI or CH₄ expressed as g/day, g/kg DMI or kJ/MJ GEI ($P > 0.05$). However, when expressed as a proportion of metabolic BW (BW^{0.75}), LUI cows had greater DMI than LIMx cows (84.5 vs. 75.7

g DMI/kg BW^{0.75}, $P < 0.05$) and produced more CH₄ per kg BW^{0.75} than LIMx cows (1.30 vs. 1.05 g CH₄/kg BW^{0.75}; $P < 0.01$). Molar proportions of acetate were higher ($P < 0.001$) and propionate and butyrate lower ($P < 0.01$) in rumen fluid samples from BG-fed compared to GS-fed cows. This study demonstrated that replacing GS with BG in barley straw-based diets can effectively reduce CH₄ emissions from beef cows, with no suppression of DMI.

Keywords: brewers' grains, cattle, greenhouse gas, methane, nutrition

Implications

Ruminant production contributes significantly to global greenhouse gas emissions. Consequently, the identification of appropriate strategies to reduce methane is becoming increasingly important. Diet formulation is one of the most promising strategies for reducing methane production from ruminants. Increasing the concentration of lipid in the diet of beef cows, by replacing grass silage with brewers' grains, reduced methane emissions by 17%. As brewers' grains are a widely available by-product feed, their use in ruminant diets provides a practical solution to reduce the environmental impact of beef enterprises.

Introduction

Ruminants play a crucial role in food security, being able to convert forages and non-human edible food into products for human consumption through enteric fermentation of cellulosic carbohydrates. However, enteric fermentation is the main source of ruminant emissions, where methane (CH₄) is an end product of the microbial digestion process. Enteric CH₄ emissions represents a loss of feed energy

to the animal (estimated at 6-10%), which could be used by the animal for production (e.g. deposition of lean meat) (Cottle *et al.*, 2011; Gerber *et al.*, 2013a and 2013b).

There is increasing interest internationally to develop sustainable approaches to reduce CH₄ production from cattle. Breeding, enterprise or system management and diet formulation are all useful strategies (Cottle *et al.*, 2011), with diet formulation representing one of the most practical and promising approaches. It has been widely demonstrated that the nutritional composition of the diet significantly affects CH₄ emissions (Cottle *et al.*, 2011). Dietary strategies to reduce CH₄ emissions are generally based on one of the following principles: (i) reducing the production of hydrogen during fermentation, (ii) direct inhibition of methanogenesis, or (iii) providing alternative pathways for the use of hydrogen within the rumen (Martin *et al.*, 2010). One promising approach, and the main focus of this paper, relates to increasing the concentration of dietary lipid which has been demonstrated to effectively reduce CH₄ emissions from ruminants (Martin *et al.*, 2010; Grainger and Beauchemin, 2011; Hristov *et al.*, 2013; Patra, 2013). Lipids reduce CH₄ emissions through various mechanisms: fatty acids are not fermented in the rumen and therefore increasing their proportion in the diet reduces the proportion of feed which is fermentable within the rumen; lipids can also reduce CH₄ production by coating fibre particles, reducing their digestibility, and by reducing the numbers and activity of the rumen methanogens and protozoa responsible for methanogenesis (Johnson and Johnson, 1995; Patra, 2013). Dietary lipid can be increased through the addition of plant oils to the diet or through the use of lipid-containing plant by-product feeds from distilleries, breweries or plant oil extraction (Brask *et al.*, 2013). The use of by-product feeds from these industries may be cost-effective and represents an important energy source in ruminant diets. Cereals used for brewing beer or distilling

spirits predominantly use the starch portion for ethanol production with the resultant by-product feed available to the ruminant feed market being proportionately higher in fibre, protein and lipid. Brewers' grains are a widely available animal feed for both beef and dairy cattle. Commonly, diets fed to housed beef cows in the winter include large proportions of forages which are low in digestibility, for example barley straw. Baseline data on CH₄ emissions from cows offered diets low in digestibility are currently sparse, as is information on effective CH₄ mitigation strategies for these diet types.

Evidence to support breed differences in CH₄ emissions is also limited. Most studies have focussed on breeds of beef cattle that are typically managed more intensively, but a small number have investigated breeds more suited to extensive grazing systems (Fraser *et al.*, 2014; Richmond *et al.*, 2015). One could speculate that breeds suited for hill and upland systems may have developed significant physiological or behavioural differences to suit the harsher environments. They may also differ in CH₄ production when offered a straw-based, poor quality diet in comparison to breeds typically managed more intensively and selected for improved growth and carcass yield.

The aim of this study was therefore to investigate the effect of increasing the concentration of dietary lipid in a barley-straw based diet, typical of industry practice, by replacing grass silage with brewers' grains, on CH₄ emissions of pregnant, non-lactating spring calving beef cows of two breeds.

Material and methods

This study was conducted at the Beef and Sheep Research Centre, SRUC situated 6 miles south of Edinburgh UK. The experiment was approved by the Animal

Experiment Committee of SRUC and was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986.

Experimental design, diets and animals

The experiment was of a two x two factorial design, comprising of two barley-straw based diets with either (i) grass silage or (ii) brewers' grains as alternative protein sources and two cow breed types (**LIMx**, crossbred Limousin; **LUI**, purebred Luings). The two experimental diets consisted (g/kg dry matter (**DM**) basis) of (i) barley straw (687) and grass silage (301; **GS**) or (ii) barley straw (763) and brewers' grains (226; **BG**), which were offered as total mixed rations (TMR). The ingredient and chemical composition of the experimental diets are given in Table 1. The chemical composition of individual dietary components is given in Table 2. The DM contents of individual components were determined twice weekly and bulked feed samples (three per component) were analysed. Feed samples were analysed for DM, ash, crude protein, acid detergent fibre, neutral detergent fibre, acid hydrolysed ether extract (AHEE), water soluble carbohydrate, starch and neutral cellulose and gamminase digestibility (Ministry of Agriculture Fisheries and Food, 1992) and gross energy by adiabatic bomb calorimetry. The LIMx cows were all Limousin-sired from a 2-breed (Limousin and Aberdeen Angus) reciprocal crossing program whilst the Luings were all purebred Luing cows. The breeds were selected to represent two commercially relevant breeds where crossbred Limousin cows represent the most common continental sired beef breed in the UK, whilst the LUI breed is typical of a more extensively managed hardy hill and upland breed.

In total 48 cows (n=24 of each breed type) were group-housed in equal numbers of each breed type across two pens, and each diet type was allocated to

one pen. Thus, 12 animals were allocated to each diet x breed combination. Treatments were balanced for age at the start of the experiment, number of days into pregnancy and BW. In the group-pens all cows were bedded on wood fibre and sawdust to ensure that consumption of bedding did not contribute to nutrient intake. Fresh water was provided *ad libitum* using a water trough, and both TMR diets were offered *ad libitum* to all cows twice daily using electronic feeders (HOKO, Insentec, Marknesse, The Netherlands). The TMR's were formulated to meet the cow's average nutrient requirements for maintenance and pregnancy according to AFRC (1993). Prior to measurements using respiration chambers, feed intake and weekly BW and body condition score (**BCS**) had been measured for a minimum of three weeks, following a four week adaptation period to acclimatise to the diets.

Emissions Measurement in Respiration Chambers

The cows were originally allocated to six respiration chambers over an eight week period, using a replicated (two times) randomised block design (each block consisting of four weeks). Thus each component of the two x two factorial (breed x diet) experimental design was allocated twice to each respiration chamber. The cows were allocated to minimise variation in stage of pregnancy and BW on entry into the respiration chambers. Prior to entry to the respiration chamber, cows were loose-housed for a period of six days individually in training pens within the same building of identical size (4 x 3 m) and shape to the pens within the respiration chambers to allow them to acclimatise to being housed individually. The cows were then moved to individual respiration chambers where they remained for three days. Cows were fed once daily with *ad libitum* access to their respective TMR's and feed consumption was monitored from weigh cells located in feed bins with records made at 10 s

intervals. Data for DMI during the 3 d chamber measurement period were averaged per animal. Front doors of chambers were briefly opened and closed at approximately 08.00 h daily to remove feed bins and again to replace bins with fresh feed at approximately 09.00 h. The pens were cleaned daily between 08.00 and 09.00 h. Exact times when doors were opened and closed were recorded.

The methodology for measuring emissions using respiration chambers has been previously described in Rooke *et al.* (2014). Briefly, six indirect open-circuit respiration chambers were used (No Pollution Industrial Systems Ltd., Edinburgh, UK). The total chamber volume (76 m³) was ventilated to give approximately 2.5 air changes/h. Temperature and relative humidity were set at 15°C and 60%, respectively. Total air flow, temperature and humidity were recorded at 5 s intervals. Chambers were operated under negative pressure (50 N/m²). Methane concentrations were measured by infrared absorption spectroscopy (MGA3000, Analytical Development Co. Ltd., Hoddesdon, UK). The analyser was calibrated before and after each three day chamber measurement period using calibration gases for zero (99.998% Nitrogen, BOC Ltd., Surrey, UK) and span (500 ppmv CH₄, 1975 ppmv CO₂, 20.9% O₂, BOC Ltd., Surrey, UK). Gas concentrations were recorded for each chamber and for inlet air every six min. Prior to the beginning of the experiment, gas recoveries were measured by releasing carbon dioxide at a constant rate into each chamber. The mean recovery was 98% (SEM 3.0) which was not different from 100%. The final 48 h of a 72 h measurement period were used to calculate daily gas production. To minimise bias caused by the entry of air when the doors to the chamber were opened for feeding, and as cows did not have access to feed at this time, gas concentrations measured during this period were not used for further analysis. Instead, and to minimise bias, these values were replaced by the

mean value of measurements made in the last hour before the doors were opened. If a cow had consumed feed during that period, mean values for the hour preceding feed consumption was used. During the 8 week period, because of failures in air recirculation (n=3) within chambers and with gas analysis (n=6), and poor DMI from one cow (n=1), data from only 38 cows were obtained and therefore an extra set of measurements were obtained in a ninth week (for 5 of 10 cows above) to bring total number of observations to 43. On completion of the experiment, critical appraisal of the data caused three of these measurements to be rejected because of gas analysis problems and therefore there were 40 observations available for analysis (n=10 for each breed x treatment combination).

Rumen sampling and volatile fatty acid analysis

Rumen fluid samples were taken from each animal within 2 h of animals leaving the respiration chambers. Animals had *ad libitum* access to feed until removal from chambers at 09.00 h (no fresh feed was provided on the morning of removal). Approximately 50 mL of rumen liquid were taken by inserting a stomach tube (16 x 2700 mm Equivet Stomach Tube, JørgenKruuse A/S, Langeskov, Denmark) nasally and aspirating manually. This liquid was filtered through two layers of muslin. A 5 mL sample of the filtered liquid was deproteinised by adding 1 mL metaphosphoric acid (215 g/L) and 0.5 ml methylvaleric acid (10 g/L) was added as an internal standard. These samples were stored at -20 °C between collection and analysis. Volatile fatty acid (VFA) concentrations were determined by HPLC as described in Rooke *et al.* (1990).

Statistical analysis

For all traits other than DMI (kg/day) statistical analyses were conducted using the mixed procedure of SAS software (SAS Inst. Inc., Cary, North Carolina). The fixed effects were diet (BG and GS) and breed (LUI and LIMx), and random effects included were week (instead of block to account for the extra week of chamber measurements) and chamber. The interaction between diet and breed was also included in the model when these effects proved significant ($P < 0.05$). For comparison of daily DMI at different measurement periods, data were analysed using the mixed procedure of SAS software using a repeated measures ANOVA including the effects of diet (BG and GS), breed (LUI and LIMx) and measurement period (group pen, training pen, chamber). There were no interactions between diet, breed and period and thus no interaction terms were included in the model. Probability values were deemed as significant where $P < 0.05$. Data are reported as means with their standard errors of the mean (**SEM**).

Results

Body weight and body condition score

Mean values for BW and BCS parameters determined in this study are presented in Table 3. Due to cow allocation to treatments there were no diet or breed differences for either age or number of days into pregnancy on entry to the chamber ($P > 0.05$). Furthermore, there were no between-diet differences for BW or BCS on entry to the chamber ($P > 0.05$). Body weight was affected by breed where LUI cows had lower BW than LIMx cows (572 vs. 668 kg; $P < 0.01$). Body condition score was affected by breed where LUI cows had poorer BCS than LIMx cows (2.5 vs. 3.1; $P < 0.001$).

Dry matter intake

No diet or breed differences were observed for DMI expressed as kg per day (Table 3; $P > 0.05$). Dry matter intake differed between measurement periods where cows had lower DMI (kg/day) within the group-pen environment compared to DMI measured in the training pens and respiration chambers (group pen = 9.10 kg/day, training pen = 9.66 kg/day, chamber = 9.88 kg/day; $P < 0.05$; SEM = 0.259). There was no interaction of measurement period with diet or breed ($P > 0.05$). However, when expressed as a proportion of metabolic BW ($BW^{0.75}$) LUI cows had greater DMI within the chambers than LIMx cows (84.5 vs. 75.7 g DMI/kg $BW^{0.75}$; $P < 0.05$).

Methane emissions

Cows offered the BG diet (Table 3) produced less CH_4 per day than GS-fed cows (131 vs. 156 g/day; $P < 0.01$). Whether expressed as g/kg DMI (13.5 vs. 16.4 g/kg DMI; $P < 0.05$) or kJ/MJ GEI (39.2 vs. 48.6 kJ/MJ GEI; $P < 0.01$) BG-fed cows produced less CH_4 than cows offered the GS diet. Luing cows consistently produced more CH_4 (g/day, g/kg DMI and kJ/MJ GEI) than LIMx cows although the breed effect was not significant ($P > 0.05$). However, when CH_4 emission was expressed as a proportion of metabolic BW, LUI cows produced more CH_4 than LIMx cows (1.30 vs. 1.05 g CH_4 /kg $BW^{0.75}$; $P < 0.01$).

Volatile fatty acid molar proportions

Molar proportions of acetate (Table 4) were higher in rumen fluid samples from cows fed BG compared to GS (769 vs. 737 mmol/mol; $P < 0.001$), while the proportions were lower for both propionate (146 vs. 162 mmol/mol; $P < 0.01$) and butyrate (65 vs. 80 mmol/mol; $P < 0.01$). The proportions of valerate did not differ between diet types ($P > 0.05$). Thus the acetate to propionate ratio was greater in cows fed the BG

than the GS diet (5.5 vs. 4.6; $P < 0.001$). There was no difference between the two breeds for volatile fatty acid molar proportions ($P > 0.05$).

Discussion

Diet effects on methane emissions

Increasing the concentration of lipid in ruminant diets reduces CH₄ emissions (Martin *et al.*, 2010; Grainger and Beauchemin, 2011; Hristov *et al.*, 2013; Patra, 2013). However, the effectiveness of dietary lipid depends on the type and amount of lipid added to the diet (Brask *et al.*, 2013; Hristov *et al.*, 2013). However, less attention has been paid to the nature of the basal diet. The present study demonstrated that incorporating brewers' grains into a straw-based diet reduced CH₄ emissions from beef cows; replacing grass silage with brewers' grains increased the lipid concentration from 20 to 37 g AHEE/kg diet DM and reduced CH₄ yield (g/kg DMI) by 17%. In recent reviews, Grainger and Beauchemin (2011) found that CH₄ yield decreased 1 g/kg DMI for every 10 g/kg DM increase in dietary lipid, and Martin *et al.* (2010) reported that CH₄ yield decreased by 3.8% with every 10 g lipid/kg DM increase. In the present study CH₄ yield decreased by 1.6 g/kg DMI or 10% for every 10 g AHEE/kg diet DM increase upon inclusion of brewers' grains which is greater than the above reports. In the present study, however, cows were observed to attempt to select brewers' grains from the mixed feed. This would result in a higher proportion of brewers' grains consumed compared to that offered. It was not anticipated that the cows would attempt to select specific dietary constituents, thus it is important to consider the potential differences in the composition of the consumed diet. To explore the potential difference in dietary lipid consumed, a corrected

estimate of the ratio of barley straw to brewers' grains was calculated based on the assumption that all refusals consisted solely of barley straw and did not contain brewers' grains. Based on 216 observations, the consumed ration was calculated as (g/kg DM basis) 334 brewers' grains and 666 straw instead of the formulated ration of 226 brewers' grains and 763 straw. Based on this corrected ratio, the lipid content of the diet would have increased to 49 g AHEE/kg diet DM compared to 37 g AHEE/kg diet DM in the formulated ration. This brings the results in line with the findings of Grainger and Beauchemin (2011) where at this corrected concentration a 1 g/kg reduction in CH₄ for every 10 g/kg increase in dietary lipid was observed. Furthermore, the effect of dietary lipid may be greater within a diet containing a high proportion of low digestible fibre such as barley straw. Martin *et al.* (2010) reported that the effects of dietary lipid were greater on a hay diet than a maize silage diet and previous findings have demonstrated greater reductions in CH₄ production on forage than concentrate-based diets (Lovett *et al.*, 2003; Troy *et al.*, 2015).

The use of by-products containing dietary lipid can be an effective strategy for reducing CH₄ emissions from cattle. Troy *et al.* (2015) investigated the addition of cold-pressed rapeseed cake to the diet of finishing beef steers and found that the addition of rapeseed cake, which is higher in lipid than brewers' grains (174 g AHEE/kg DM), to a mixed forage and concentrate diet (52 g AHEE/kg diet DM) resulted in a reduction in CH₄ yield of 3.3% (0.83 g/kg DMI) per 10 g/kg DM increase in dietary lipid, which is slightly lower than the results reported here. Brask *et al.*, (2013) added rapeseed cake (173 g crude fat/kg DM) to the diet of dairy cows and found a greater CH₄ yield reduction of 4.6% for every 10 g/kg DM increase in dietary lipid. Relatively few studies have reported the effects of including brewers' grains in the diet on CH₄ production. However, Moate *et al.* (2011) used brewers' grains,

hominy meal and a combination of hominy meal and cold pressed rapeseed in dairy cow diets, where the diets contained 51, 65 and 52 g crude fat/kg diet DM, respectively (compared to the control which contained 26 g crude fat /kg diet DM). Moate *et al.* (2011) observed a 5% reduction of CH₄ yield on both the brewers' grains and combined hominy meal and rapeseed treatments, and 12% on the hominy meal treatment; the greater reduction on the hominy meal treatment was likely due to the higher lipid concentration in the diet. They demonstrated for each 10 g/kg DM increase in dietary lipid concentration, CH₄ emissions were reduced by 3.5%. Although the majority of studies to date have not investigated the persistency of the effects of lipid on suppressing CH₄ production, Moate *et al.* (2011) demonstrated a persistency of their dietary effects over more than 7 weeks. The effect of lipid persisted throughout the current experiment (9 weeks) as there was no effect of measurement week ($P = 0.50$) on CH₄ production.

One of the mechanisms by which lipid is thought to suppress CH₄ production is through increased production of propionate versus acetate and thus reduction in the amounts of hydrogen generated through fermentation. The meta-analysis of Patra (2013) demonstrated that although total VFA concentrations were not altered by increasing the dietary lipid content, the proportion of propionate to acetate increased and the proportion of butyrate decreased with increasing concentration of lipid which supports the above mechanism. In contrast, an increase in acetate and decrease in propionate on the BG diet was observed in the present study alongside a reduction in butyrate. Increasing the concentrate proportion of the diet is normally associated with increases in propionate molar proportions, although the response is likely to depend on the nutrients supplied by the diet. In the present study, 964 g/kg DM in diet BG was accounted for by neutral detergent fibre, crude protein, AHEE

and ash whereas only 649 g/kg DM was accounted for by these constituents in diet GS. The constituents unaccounted for in GS include fermentation acids, particularly lactic acid. Since there is a positive correlation between silage lactic acid concentration and rumen propionic acid molar proportion (Martin *et al.* 1994), the greater propionic acid molar proportions in the GS diet most likely reflected the lactic acid in the silage and the low concentrations of starch in the brewers' grains. From the above, decreased hydrogen supply to the rumen archaea from increased production of propionic acid does not appear to be the most likely mechanism of action of lipid in the present experiment. More likely mechanisms may be physical coating of fibre by the lipid and the reduction in rumen-fermentable substrates as a result of lipid addition. Furthermore, apart from the increased lipid content of the diet, the other main changes in the composition of the diet when brewers' grains replaced grass silage were increased NDF and CP contents. The greater acetate to propionate ratio observed for diet BG is consistent with the increase in NDF but there was no increase in branched chain VFA on diet BG which might be expected from increased protein degradation. However, the increase in acetate to propionate ratio on diet BG was not associated as might be expected with an increase in CH₄ emissions and therefore it is likely that the increase in dietary lipid was the major factor underlying the reduction in CH₄ emissions observed when BG rather than GS was fed.

At high concentrations in the diet, lipid can negatively affect DMI and productivity, but low concentration of dietary lipid can be used with no adverse effects (Brask *et al.*, 2013). Based on a meta-analysis, Patra (2013) demonstrated that lipid supplementation in excess of 6% causes problems with productivity. Diets which negatively affect productivity are unsuitable for livestock producers, and

therefore, in the present study the concentration of lipid in the BG diet was 37 g AHEE/kg diet DM in the formulated ration. As expected, and consistent with the literature, this concentration of dietary lipid did not suppress DMI. Even if we assume the animals preferentially selected the diet as observed, the diet would still not have reached a concentration of lipid expected to suppress DMI. However, at this corrected level of BG inclusion a marked decrease in CH₄ production was observed without adverse effects on DMI. Therefore, the use of by-products such as brewers' grains, represents an attractive strategy for use in beef cow diets from both an animal productivity and CH₄ mitigation perspective.

In the present study mean CH₄ yields for each of the BG and GS diets were 0.039 and 0.049 MJ/MJ GEI respectively, considerably lower than the value currently adopted by the IPCC (2006) (0.065 MJ/MJ GEI). The IPCC (2006) approach does not account for differences in the digestibility of diets and as a result over-estimates CH₄ yield from diets containing large proportions of forages which are low in digestibility.

Respiration chambers are generally considered to be the most accurate technology for measuring CH₄ emissions from ruminants. However, one of the major challenges associated with this technology is avoiding a reduction in feed intake within the chamber environment, where the animals are individually housed. This is necessary for CH₄ emissions data to be representative of normal feeding behaviour in a group-housed environment (Garnsworthy *et al.*, 2012; Bickell *et al.*, 2014). In the present study, no reduction in DMI was observed from group-housing to respiration chambers, but there was a small increase in DMI per day of 9%.

Breed effects on methane emissions

There is limited experimental evidence to support differences in CH₄ emissions between breeds. Rooke *et al.* (2014) examined CH₄ emissions from crossbred Limousin and crossbred Aberdeen Angus, and found no difference between these breeds in methane yield whether expressed per level of DMI or GEI. Troy *et al.* (2015) compared two breeds of finishing beef steers (purebred Luing and crossbred Charolais) and reported no differences between breeds in CH₄ whether expressed as g/day, g/kg DMI or kJ/MJ GEI. These studies were both conducted using the same respiration chambers and methodologies to those used in the current study.

Differences in grazing behaviour between breeds is likely to have a large impact on CH₄ emissions, as demonstrated in a modelling study by Ricci *et al.* (2014). Based on the potential for differences in animal physiology or behaviour to influence CH₄ production, a number of recent studies have been conducted within outdoor grazing environments to examine CH₄ emissions from breeds which are suited to extensive grazing systems compared to more intensively managed breeds selected for increased growth and carcass yields. Measurements of CH₄ in grazing environments are possible using the SF₆ tracer technique (Deighton *et al.*, 2014). In the studies of Fraser *et al.* (2014) and Richmond *et al.* (2015), where two breeds were studied on two pasture types (lowland vs. upland pasture), no difference between breeds (or interactions with pasture type) were identified for CH₄ expressed on a daily, DMI or GEI basis. Rooke *et al.* (2015) reported CH₄ emissions of lactating beef cows of the same two breeds considered in the present study on either reseeded predominantly perennial ryegrass pasture or rough hill grazing. Consistent with previous findings, daily CH₄ emissions were influenced by pasture type, but not breed. The results of the present study, although measured in chamber environments, were consistent with Rooke *et al.* (2015) where these breeds of beef

cow did not influence CH₄ when expressed as g/day, g/kg DMI or kJ/MJ GEI. These expressions of CH₄, however do not take account of the differences in BW of these two breed types. Within the present study, LUI cows were considerably smaller than the LIMx cows (572 vs. 668 kg BW) and produced greater levels of CH₄ per kg metabolic BW compared to the LIMx cows. This greater level of CH₄ is driven by the differences in DMI/kg BW^{0.75}, where LUI cows consumed greater DMI per kg metabolic BW than LIMx cows. When considering the difference between breeds, it is important to take additional characteristics of the animals (such as DMI/kg BW^{0.75}) into account as these have an important influence on CH₄ production.

Increasing the dietary lipid concentration has been shown to effectively reduce CH₄ production from ruminants provided the amount fed is less than that which adversely affects digestion and feed intake. However, the practicality and sustainability of this approach is dependent on the type of lipid used. Pure oils that could be used for human food are of high cost, or indeed where production is controversial (palm oil) may not represent the best sustainable solution. The use of by-products from ethanol production (biodiesel and alcoholic beverages) or oil extraction produces feeds that are better balanced in protein and lipid/energy supply than the parent feeds and are well established for use within ruminant diets. Thus the use of by-products, such as brewers' grains represents a cost-effective and sustainable solution for mitigation of CH₄ from ruminant systems.

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Table 1 Component composition and calculated chemical composition of experimental diets
(BG, Barley straw- brewers' grains; GS, Barley straw-grass silage)

Diets	BG	GS
Components (g/kg dry matter)		
Barley straw	763	687
Grass silage		301
Brewers' grains	226	
Mineral / vitamin mix ¹	11	12
Composition (g/kg dry matter)		
Dry matter (g/kg)	550	533
Crude protein	73	59
NDF ²	771	693
ADF ²	516	499
Starch	6	0
WSC ²	6	20
AHEE ²	37	20
Ash	38	48
Metabolisable energy (MJ /kg DM)	7.4	8.1
Gross energy (MJ /kg DM)	19.4	18.9

¹mineral / vitamin mix (Norvite, Inch, Aberdeenshire, UK) supplied (mg /kg unless stated otherwise) vitamin A, 500000 international units (IU); Vitamin D 100000 iu; Vitamin E 4000; Fe, 5271; Mn, 5000; Zn, 3600; I, 1000; Co, 90; Cu, 3000; Se, 35.

²NDF, neutral detergent fibre; ADF, acid detergent fibre; WSC, water soluble carbohydrate; AHEE, acid hydrolysed ether extract

568 **Table 2** *Chemical composition of components (g/kg DM)*

	Barley straw	Grass silage	Brewers' grains
Dry matter (g/kg)	805	298	263
Crude protein	20	150	255
NDF ¹	847	370	553
ADF ¹	593	303	279
Starch	0	0	26
WSC ¹	7	50	3
AHEE ¹	14	36	118
Ash	38	73	38
NCGD ¹	308	0	567
Metabolisable energy (MJ /kg DM)	6.5	12.1	10.9
Gross energy (MJ /kg DM)	18.8	19.8	22.5
pH		4.3	

569 ¹NDF, neutral detergent fibre; ADF, acid detergent fibre; WSC, water soluble carbohydrate;
570 AHEE, acid hydrolysed ether extract; NCGD, neutral cellulose and gamanase digestibility

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Table 3 Age, BW and body condition score (BCS) of cows at allocation, intakes and CH₄ production as measured from the respiration chambers (means with average SEM)

Diet ¹	BG		GS		SEM	Significance ⁴	
Breed	LIMx	LUI	LIMx	LUI		Breed	Diet
On entry to chamber							
Age (years)	5.2	5.7	6.1	4.6	1.33	ns	ns
Days pregnant ²	212	224	226	212	4.11	ns	ns
BW (kg)	697	587	638	557	32.58	**	ns
BCS	3.2	2.6	3.0	2.4	0.12	***	ns
DMI							
Group pen kg/d ³	9.62	8.65	9.28	8.84	0.60	ns	ns
Training pen kg/d	9.70	9.36	10.32	9.26	0.60	ns	ns
Chamber kg/d	10.06	9.77	9.90	9.81	0.82	ns	ns
Chamber g/kg BW ^{0.75}	74.1	82.8	79.0	85.8	6.45	*	ns
CH ₄							
g/d	129	133	143	169	11.17	ns	**
g/kg DMI	13.2	13.9	14.7	18.0	1.48	ns	*
kJ/MJ GEI	38.2	40.3	43.8	53.4	4.36	ns	**
g/kg BW ^{0.75}	0.95	1.12	1.14	1.48	0.09	**	***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

DMI, dry matter intake; GEI, gross energy intake; LIMx, crossbred Limousin; LUI, purebred Luing

¹BG, Barley Straw-Brewers' Grains; GS, Barley Straw-Grass Silage

²Three animals were identified as not in calf (all LUI, one allocated to BG and 2 allocated to GS diet)

³Measured throughout 1 week prior to entry to training pen

⁴The interaction effect of breed x diet was not significant for any trait except days pregnant ($P < 0.01$).

Table 4 Volatile fatty acid molar proportions (mmol/mol) in rumen fluid samples taken on exit from respiration chambers (means and average SEM are given for effects of breed and diet)

Diet ¹	BG		GS		SEM	Significance ³	
Breed	LIMx	LUI	LIMx	LUI		Breed	Diet
Acetate	769	770	738	736	9.2	ns	***
Propionate	145	146	162	162	9.0	ns	**
Butyrate	65	65	78	81	4.2	ns	***
Valerate	6	6	6	6	0.6	ns	ns
Branched Chain ²	15	13	16	15	1.2	ns	ns
Acetate:propionate ratio	5.5	5.4	4.6	4.6	0.4	ns	***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

LIMx, crossbred Limousin; LUI, purebred Luining

¹BG, Barley Straw-Brewers' Grains; GS, Barley Straw-Grass Silage

²Branched chain is sum of iso-butyrate and iso-valerate

³The interaction effect of breed x diet was not significant for any trait